Anopheles lindesayi japonicus Yamada (Diptera: Culicidae) in Korea: comprehensive review, new collection records, and description of larval habitats

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Received 13 September 2007; Accepted 29 December 2007

ABSTRACT: *Anopheles lindesayi japonicus* Yamada is an uncommonly collected mosquito in Korea, and its presence is based upon limited collection data and anecdotal reports in Korean mosquito literature: 45 specimens collected from 15 identified sites. This study reports the collection of 538 specimens from 16 sites as part of the authors' 2004-2007 anopheline surveillance. Larvae were collected from stream margins, stream pools, rock pools, seepage springs, artificial containers, swamps, and ditches and were found in association with seven other culicid species. Inclusion of the authors' data with previous published and unpublished records makes this a comprehensive report on this species in Korea. New province records are reported for this species at Hwacheon and Wonju in Gangwon Province, Mt. Palgong in Daegu Metropolitan, and Chungju and Mt. Worak in Chungcheongbuk Province in the Republic of Korea. ***Journal of Vector Ecology*** **33 (1): 99-106. 2008.**

Keyword Index: Korea, mosquito, *Anopheles*, *lindesayi*, *japonicus*, distribution.

INTRODUCTION

Reid and Knight (1961) recognized the *Anopheles lindesayi* Complex as being composed of three species: *An. gigas* Giles, *An. lindesayi* Giles, and *An. wellingtonianus* Alcock. The species *An. lindesayi* is reported throughout much of Asia from India, China, Japan, Korea, Malaysia, the Philippines, Taiwan, (Reid 1968), Thailand (Harrison et al. 1991), and Afghanistan and Pakistan (Glick 1992). This species has been divided further into subspecies, with some authors recognizing five subspecies (Christophers 1933, Tanaka et al. 1979) and one author recognizing six subspecies (Reid 1968). The 2001 Systematic Catalog of Culicidae posted and maintained on the Walter Reed Biosystematics webpage (http://www.mosquitocatalog.org) lists only four subspecies: *japonicus* Yamada, *pleccau* Koidzumi, *cameronensis* Edwards, and *benguetensis* King. The former fifth subspecies, *An. nilgiricus* Christophers, was elevated to full species by Harrison et al. (1991).

Limited ecological and zoogeographic data for the currently recognized subspecies of *An. lindesayi* suggest that they represent isolated populations of an ancestral species that was previously widespread in cool temperate areas of Asia, including the Indian Subregion. The climatic conditions and ocean levels during earlier periods allowed the ancestral species to have a widespread continuous distribution at lower elevations. Subsequently, with climatic warming and rising ocean levels, populations of the ancestral species evolved into the current recognized subspecies due to either insular or allopatric isolation on the tops of mountains and at more northern latitudes. Harrison et al. (1991) stated that the members of the Lindesayi Complex, including *An. lindesayi* and subspecies, *An. mengalangensis* Ma, *An. nilgiricus* Christophers, and *An. wellingtonianus* Alcock, (see Harbach 2004), exhibit most of the attributes of a superspecies (Mayr and Ashlock 1991), i.e., they are a monophyletic group of closely related and largely or entirely allopatric species. Earlier discussions regarding taxa recognized under the name *An. lindesayi* can be found in Christophers (1933) and Reid (1968). Potentially, the subspecies of *An. lindesayi* may be elevated to species level, with primary emphasis on molecular analysis because the previously described morphological characters are polymorphic.

The subspecies, *Anopheles lindesayi japonicus*, is known from multiple islands of Japan and detailed surveillance data on its distribution and bionomics are available beginning with LaCasse and Yamaguti (1950). It is the only subspecies of this complex reported from Korea. Detailed descriptions of the female, male, and larva of this species and its subspecies

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
SEP 2007		**00-00-2007 to 00-00-2007**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Anopheles lindesayi japonicus Yamada (Diptera: Culicidae) in Korea: comprehensive review, new collection records, and description of larval habitats	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Walter Reed Army Institute of Research,Department of Entomology,503 Robert Grant Avenue,Silver Spring,MD,20910**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
see report

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**8**	

may be found in several sources: Christophers (1933), LaCasse and Yamaguti (1950), Reid (1968), and Tanaka et al. (1979); pupal and spiracular setae descriptions are found in Ohmori (1959 and 1960, respectively). *Anopheles l. japonicus* is not implicated in the transmission of malaria or any other diseases.

In May 1961, Whang (1962) collected two *An. l. japonicus* from a cowshed in Gidan-dong, Yean-myeon, Andong, Gyeongsangbuk Province, and in May 1962, Hong and Ree (1968) reported a second collection in Gyeonggi Province. These were the first two published reports on this species in Korea. Other reports followed (Dubar 1965, Kimbell 1966, Lien 1969, Lee 1971, Tanaka et al. 1979, Egan 1985, Lee 1994, Kim et al. 2003, Rueda et al. 2006), but citations of *An. l. japonicus* collections were usually anecdotal to the primary study focus of those authors. Lien (1969), however, reported the examination of *An. l. japonicus* specimens from U.S. Army workers in 1945-1950, but no written documentation was found on these data. He also reported examining specimens from Korean and U.S. Army workers for the period 1962-1968. It is unclear as to the source of these data, but his report suggests that specimens were being collected and preserved in some manner. In a similar fashion to other reports of *An. l. japonicus*, the current authors found populations of *An. l. japonicus* as part of other anopheline surveillance and initiated investigations into the documentation and occurrence of this species in Korea.

MATERIALS AND METHODS

In 2004-2007, the authors used a variety of surveillance methods to identify the specific habitats of anopheline species comprising the *Anopheles sinensis* complex in order to determine the larval habitat of each species within the complex. Methods included aspiration of resting mosquitoes, dipping and siphoning larval collections with detailed habitat descriptions and photographs of each site, and adult collections using New Jersey Light Traps (NJLT), Centers for Disease Control (CDC) light traps, and Mosquito Magnets® (Pro Model, American Biophysics Corp., Greenwich, RI). Incidental to the conduct of these surveys, both adult and larval specimens of *An. l. japonicus* were collected.

Collected larvae and/or pupae were reared to the adult stage (LPRA) for identification. The broad palmate setae on *An. l. japonicus* larvae are readily visible to the unaided eye and makes the larvae of this species distinguishable from all other larval anophelines in Korea. Therefore, authors could readily field identify collected *An. l. japonicus* larvae and separate them for further rearing.

For future comparisons with *An. l. japonicus* populations in other countries, reared adults from this study were used to determine the molecular species identification as described by Wilkerson et al. (2003). Hence, genomic DNA was extracted from single legs of *An. l. japonicus*. The ITS2 primers from the 5.8S and 28S coding regions flanking the ITS2 region were used to amplify the genomic DNA. Reactions were carried out in a total volume of 20 ul using PCR premix (Bioneer, Korea). The amplification profile was denaturation at 95° C for 10 min, 35 cycles of 30 s at 94° C, 1 min at 60° C, 1 min at 72° C, followed by a final extension at 72° C for 5 min. The PCR products were separated on a 2% agarose gel and visualized with ethidium bromide stain. Fragment sizes were estimated by comparison to molecular weight standards provided by 100bp Ladder Molecular Weight DNA Marker (Intron Biotechnology, Korea).

RESULTS AND DISCUSSION

We compiled the 2004-2007 collection data on *An. l. japonicus* with previous published and unpublished records to make this a comprehensive report on this species in Korea (Figure 1). Korean data were also discussed in relation to *An. l. japonicus* data from Japan, due in part to its proximity and to the amount of data reported from that country.

Prior to this study, 45 *An. l. japonicus* from 15 identified sites (an additional four studies did not provide adequate information to determine a specific location other than province) had been reported from Korea from all sources (Whang 1962, Dubar 1965, Kimbell 1966, Hong and Ree 1968, Lien 1969, Lee 1971, Tanaka et al. 1979, Egan 1985, Lee 1994, Kim et al. 2003, Rueda et al. 2006) (Table 1). The current study resulted in an additional 538 specimens (adults as well as LPRA) from 16 larval sites including new province and metropolitan records for Gangwon Province, Daegu Metropolitan, and Chungcheongbuk Province. At one collection site, *An. l. japonicus* adults were collected on five separate dates between July and September 2006 (Table 2). There was also one larval site near Munsan where multiple collections on different dates (2004-2007) were made from stream pools along the same stream, and larval sites of Mt. Palgong, Mt. Worak, and Chungju areas where collections at different sites were made from stream pools and stream margins along the same stream.

The authors were not able to find and read the original reports by Dubar (1965) and Kimbell (1966); however, Barrett's (1969) summary report incorporated some of the data from the Dubar and Kimbell reports. Barrett's report stated that one adult *An. l. japonicus* specimen was collected each year, but he did not mention the sex of the specimen or the location of the collection.

Anopheles l. japonicus larvae collected in this study were found at elevations of 25-384 m (Table 2). Similarly for Japan, LaCasse and Yamaguti (1950) reported collecting *An. l. japonicus* in increasing frequency at greater elevations up to elevations of 305-610 m. Others (Christophers 1933, Reid 1968, Tanaka et al. 1979, Harrison et al. 1991) have reported that other subspecies of *An. lindesayi* were collected at much higher elevations.

Larvae in this study were found to be in association with larvae of: *An. koreicus* Yamada and Watanabe, *An. sinensis* Wiedemann, *An. sineroides* Yamada, *Culex hayashii* Yamada, *Cx. orientalis* Edwards, *Cx. pipiens* L., and *Cx. rubensis* Sasa and Takahashi (Table 3). In comparison, LaCasse and Yamaguti (1950) found *An. l. japonicus* in Japan to be in association with larvae of: *An. koreicus*, *An. sinensis*,

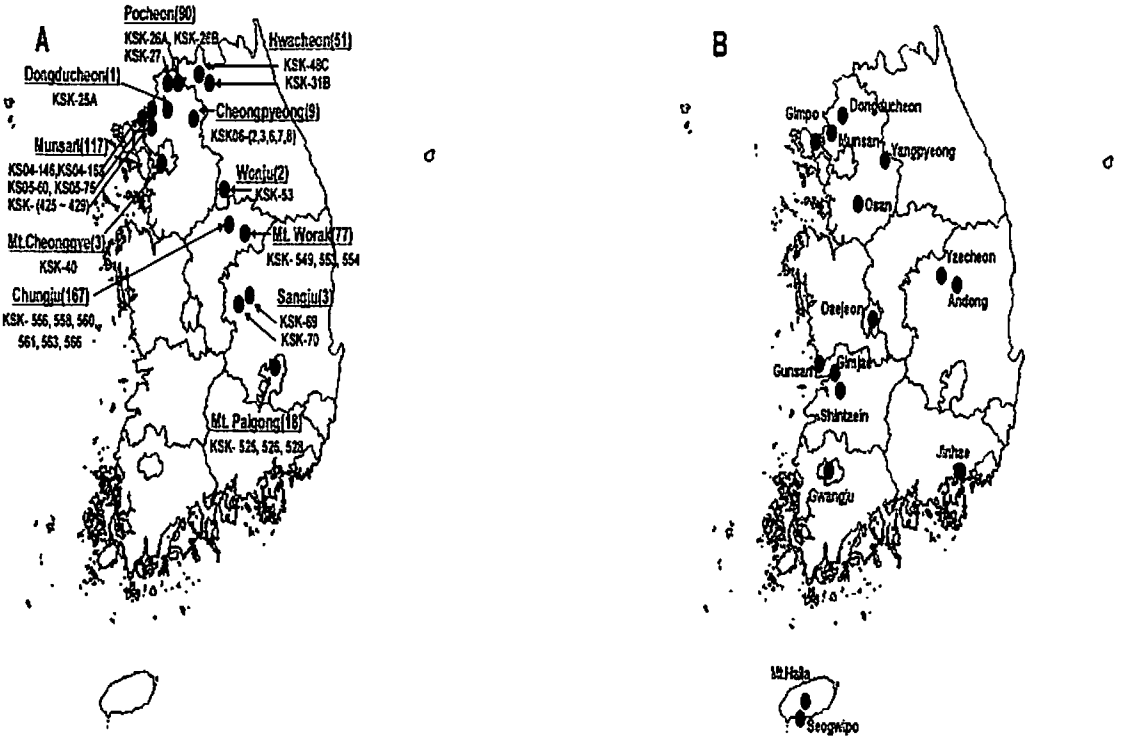

Figure 1. Maps showing collection sites of *Anopheles lindesayi japonicus* reported in this study, (A) 2004-2007 collection sites and numbers (number collected), and in previous reports, (B) 1961-2003.

An. sineroides, *Cx. hayashii*, *Cx. infantulus* Edwards, *Cx. mimeticus* Noe, *Cx. orientalis*, *Cx. pipiens*, *Cx. ryukyensis* Bohart, *Cx. tritaeniorhynchus* Giles, *Lutzia vorax* (Edwards) (reported as *Culex* in LaCasse and Yamaguti (1950) but moved to *Lutzia* by Tanaka (2003)), *Orthopodomyia anopheloides* (Giles) (reported as *Or. nipponica* in LaCasse and Yamaguti (1950), but Knight and Mattingly (1950) placed *nipponica* as a synonym of *anopheloides*), and *Tripteroides bambusa* (Yamada).

In Japan, LaCasse and Yamaguti (1950) reported the following as collection sites for *An. l. japonicus*: bowls (cement, glass, metal, earthenware, or stone), buckets (metal or wood), tanks (cement for emergency firefighting), vases (earthenware or stone), stationary tanks and excavations for garden irrigation or fire fighting, ground pools, ponds (natural and artificial impoundments), rice fields (planted and neglected paddies), roadside ditches, field drainage and irrigation ditches, street and roadside gutters, and along slowly moving streams. They also stated that *An. l. japonicus* was taken "...mostly from the vegetated margins of slow moving streams, fresh water rain pools containing green algae, and garden irrigation tanks containing fresh water." For Tanegashima and Yakushima Islands, Mogi (1996) reported finding *An. l. japonicus* larvae in a spring.

For Jeju Island (Korea), Lee (1994) reported finding *An. l. japonicus* along ground and stream pools and in rock holes or cavities containing fresh water. This represents the only reported larval habitat description for Korea prior to this study.

Herein, the authors characterized the larval habitats of *An. l. japonicus* into seven categories of larval breeding sites: stream pool (256 *An. l. japonicus* larvae), stream margin (109), rock pool (89), seepage-spring (59), artificial container (11), swamp (3), and ditch (2) (Table 2). Generally, authors noted that *An. l. japonicus* larvae were found along slow to moderate flowing streams with isolated pools that were not apart of the main stream. These isolated pools were primarily from seepage springs and rock pools that were filled by spring flow or splashing action from falls or cascades, respectively, with rock holes also being affected by rainfall. Stream margins and stream pools also provided good habitats for *An. l. japonicus* larvae, especially if vegetation from the surrounding land overflowed into the water, if the embankment provided a form of cover for the developing larvae, or if surface algae were present. The land surrounding the streams was moderately- to steeply-sloping shaded hills/mountains covered in a mix of deciduous and conifer (primarily pine) forests.

At Mt. Worak, larvae were collected in great abundance (>100 larvae) along the protected margins of streams where water flow was minimal and there was floating debris (dead vegetation) and/or vegetation hanging over the margin edge. Similar numbers were collected in larger pools associated with the stream margin, with fewer numbers collected in smaller pools. As the stream broadened into a narrow valley, other species of *Anopheles* predominated. Additionally, rock pools along the same stream where *An. l. japonicus* larvae were collected only yielded *Aedes* spp. and *Armigeres subalbatus*, indicating that they are not a preferred habitat. This provides evidence that the preferred habitat of *An. l. japonicus* is shaded mountain stream margins and pools (considering the numbers collected), with the potential for

Table 1. Data for *Anopheles lindesayi japonicus* in the Republic of Korea 1961-2003 as reported in published literature and reports.

Data Source[1]	Date Collected	Location	Province	Methods[2]	Habitats[3]	Total No. Collected[4]
Whang 1962	May 1961	Guidan-dong,Yean-myeon, Andong	Gyeongsangbuk	Aspirator	Cowshed	2-F
Dubar 1965	1965	?	?	NJLT		1
Kimbell 1966	1966	?	?	NJLT		1
Hong & Ree 1968	May 1962	Gaegun-myeon, Yangpyeong-gun	Gyeonggi			1-F
Lien 1969	Aug-Sep 1969	Shintaein	Jeollabuk	L		1-F, 1-M
		Seoguipo, Namjeju-gun	Jeju	L		
Lee 1971	?	Geumsan Temple, Gimjae	Jeollabuk	L		?
Biery & Burns 1973	Sep 1970	Gimpo Air Base (Seoul)	Gyeonggi	NJLT		1
Biery & Burns 1973	1970-1971	Gunsan Air Base	Jeollabuk	NJLT		6
Biery & Burns 1973	1970-1972	Gwangju Air Base	Jeollanam	NJLT		5
Biery & Burns 1973	1970-1972	Osan Air Base	Gyeonggi	NJLT		3
Hong 1977	1968	Yongmun Temple, Yaecheon-gun	Gyeongsangbuk	L	Unused W/C	18
Tanaka *et al.* 1979	7 Sep 1971	Mt. Halla	Jeju	L	RP	1-F, 1-M
Egan 1985	Jul 1984	Camp Ames (Daejeon)	Chungcheongnam	NJLT		1-F
Lee 1994	?	?	Jeju	L	GP, RP, SP	?
Ko 1996	?	?	Jeju			?
Miller and O'Brien 1998	10 Jul 1998	Jinhae Naval Base	Gyeongsangnam	CDC LT		1-F
Kim et al. 2003[5]	29 Sep 1999	Camp Hovey (Dongducheon)	Gyeonggi	NJLT		1-F
TOTAL						45

[1]Published journal article or report from which the information was extracted.
[2]CDC LT=Centers for Disease Control Light Trap; NJLT=New Jersey Light Trap; L=Larval collection.
[3]W/C=well/cistern; GP=ground pool; RP=rock pool/hole; SM=stream margin; SP=stream pool.
[4]Larval collections were reared to the adult stage for these data. Lee (1994) collected an unspecified number of larvae. F=females, M=males for those sources who reported the sex of their specimens. Aspirator, NJLT, and CDC LT were all adult collections.
[5]The same specimen is also referred to in Rueda et al. 2006.

Table 2. Data for *Anopheles lindesayi japonicus* collected in the Republic of Korea 2004-2007.

Serial No.[1]	Date Collected	Grid Coordinates	Locality/Province	Habitats[2]	Altitude (meters)	Total No. Collected[3]	Female	Male
KS04-146	24-Jul-04	37° 53' 37" N/126° 49' 33" E	7 km W Munsan along Hwy 37/ Gyeonggi	SP	54	2 (L)	2	0
KS04-163	02-Sep-04	37° 53' 37" N/126° 49' 33" E	7 km W Munsan along Hwy 37/ Gyeonggi	SP	54	2 (L)	2	0
KS05-60	24-Aug-05	37° 53' 38" N/126° 49' 32" E	7 km W Munsan along Hwy 37/ Gyeonggi	SP	27	1 (L)	1	0
KS05-75	31-Aug-05	37° 53' 38" N/126° 49' 32" E	7 km W Munsan along Hwy 37/ Gyeonggi	SP	27	18 (L)	8	10
KSK-25A	29-Sep-06	37° 55' 25" N/127° 04' 30" E	Dongducheon/Gyeonggi	SP	100	1 (L)	1	0
KSK-26A	29-Sep-06	37° 57' 46" N/127° 15' 41" E	Hwy 37 approx 1 km east of Hwy 43/ Gyeonggi	SS	92	42 (L)	29	13
KSK-26B	29-Sep-06	37° 57' 46" N/127° 15' 41" E	Hwy 37 approx 1 km east of Hwy 43/ Gyeonggi	SS	92	17 (L)	10	7
KSK-27	29-Sep-06	37° 55' 25" N/127° 15' 37" E	Jihyeon/Gyeonggi	SM	150	31 (L)	17	14
KSK-31B	29-Sep-06	38° 03' 15" N/127° 34' 14" E	Hwy 56 E of Sachang-ri, Hwacheon-gun/ Gangwon	AC	207	11 (L)	5	6
KSK-40	08-Oct-06	37° 26' 25" N/127° 03' 05" E	Mt. Cheonggye/Gyeonggi	RP	119	3 (L)	3	0
KSK-48C	18-Oct-06	38° 05' 07" N/127° 31' 37" E	Sanaemyun, Hwacheon-gun/ Gangwon	RP	331	40 (L)	25	15
KSK-53	20-Oct-06	37° 24' 23" N/127° 57' 23" E	Taejangdong, Wonju-si/Gangwon	D	118	2 (L)	0	2
KSK-69	25-Oct-06	36° 28' 43" N/128° 00' 16" E	Imgok-ri, Hwaseo-myeon,Sangju-si/ Gyeongsangbuk	S	257	2 (L)	1	1
KSK-70	25-Oct-06	36° 26' 30" N/127° 53' 12" E	Imgok-ri, Hwaseo-myeon,Sangju-si/ Gyeongsangbuk	S	231	1 (L)	0	1
KSK-06-2	21-Jul-06	37° 39' 59" N/127° 23' 11" E	Cheongpyeong/Gyeonggi	Cowshed	46	1 (A)	1	0
KSK-06-3	25-Jul-06	37° 39' 59" N/127° 23' 11" E	Cheongpyeong/Gyeonggi	Cowshed	46	1 (A)	1	0
KSK-06-6	28-Aug-06	37° 39' 59" N/127° 23' 11" E	Cheongpyeong/Gyeonggi	Cowshed	46	1 (A)	1	0
KSK-06-7	12-Sep-06	37° 39' 59" N/127° 23' 11" E	Cheongpyeong/Gyeonggi	Cowshed	46	5 (A)	5	0
KSK-06-8	20-Sep-06	37° 39' 59" N/127° 23' 11" E	Cheongpyeong/Gyeonggi	Cowshed	46	1 (A)	1	0
KSK-425	10-Sep-07	37° 53' 28" N/126° 49' 42" E	7 km W Munsan along Hwy 37/ Gyeonggi	SP	25	29 (L)	14	15
KSK-426	10-Sep-07	37° 53' 28" N/126° 49' 42" E	7 km W Munsan along Hwy 37/ Gyeonggi	SP	25	33 (L)	17	16
KSK-427	10-Sep-07	37° 53' 28" N/126° 49' 42" E	7 km W Munsan along Hwy 37/ Gyeonggi	SP	25	19 (L)	11	8
KSK-428	10-Sep-07	37° 53' 28" N/126° 49' 42" E	7 km W Munsan along Hwy 37/ Gyeonggi	SP	25	3 (L)	1	2
KSK-429	10-Sep-07	37° 53' 28" N/126° 49' 42" E	7 km W Munsan along Hwy 37/ Gyeonggi	SP	25	10 (L)	1	9
KSK-525	14-Oct-07	35° 58' 15" N/128° 46' 39" E	Mt. Palgong/ Daegu	SM	198	3 (L)	1	2
KSK-526	14-Oct-07	35° 58' 15" N/128° 46' 34" E	Mt. Palgong/ Daegu	SM	226	10 (L)	5	5
KSK-528	14-Oct-07	35° 58' 15" N/128° 46' 34" E	Mt. Palgong/ Daegu	SM	198	5 (L)	2	3
KSK-549	16-Oct-07	36° 55' 21" N/128° 13' 36" E	Mt. Worak/ Chungcheongbuk	SM	154	1 (L)	1	0
KSK-553	16-Oct-07	36° 55' 18" N/128° 13' 30" E	Mt. Worak/ Chungcheongbuk	RP	161	46 (L)	31	15
KSK-554	16-Oct-07	36° 55' 16" N/128° 13' 26" E	Mt. Worak/ Chungcheongbuk	SM	172	30 (L)	17	13
KSK-556	16-Oct-07	36° 55' 07" N/127° 56' 38" E	7 km W Chungju along Hwy 36/ Chungcheongbuk	SP	87	21 (L)	10	11
KSK-558	16-Oct-07	36° 55' 07" N/127° 56' 38" E	7 km W Chungju along Hwy 36/ Chungcheongbuk	SP	87	24 (L)	13	11
KSK-560	16-Oct-07	36° 44' 17" N/127° 54' 19" E	5 km S Chungju along Hwy 517/ Chungcheongbuk	SP	237	37 (L)	10	27
KSK-561	16-Oct-07	36° 44' 22" N/127° 54' 20" E	5 km S Chungju along Hwy 517/ Chungcheongbuk	SM	230	29 (L)	11	18
KSK-563	16-Oct-07	36° 44' 28" N/127° 54' 20" E	7 km S Chungju along Hwy 517/ Chungcheongbuk	SP	227	5 (L)	4	1
KSK-566	16-Oct-07	36° 44' 51" N/127° 54' 42" E	7 km S Chungju along Hwy 517/ Chungcheongbuk	SP	384	51 (L)	30	21
TOTAL						538	292	246

[1]Collection numbers used by authors. [2]AC=artificial container; D=ditch; RP=rock pool; S=swamp; SP=stream pool; SS=seepage spring; SM=stream margin. [3]L=larval collection; A=adult collection. For larval collections, the numbers represent the number of adults that were reared from larvae and/or pupae.

Table 3. Larval mosquito species reported in association with *Anopheles lindesayi japonicus* in Korea and Japan.

Species[1]	Species Known to Occur in Korea	Associated with *An. l. japonicus* in Korea[3]	Species Known to Occur in Japan[4]	Associated with *An. l. japonicus* in Japan
An. koreicus	X	X	X	X
An. sinensis	X	X	X	X
An. sineroides	X	X	X	X
Cx. hayashii	X	X	X	X
Cx. infantulus	X		X	X
Cx. mimeticus	X		X	X
Cx. orientalis	X	X	X	X
Cx. pipiens	X	X	X	X
Cx. ryukyensis			X	X
Cx. rubensis	X	X	X	
Cx. tritaeniorhynchus	X		X	X
Lt. vorax[2]			X	X
Or. anopheloides[2]			X	X
Tr. bambusa	X		X	X

[1] List is a union of species in Korea and Japan that are associated with *An. l. japonicus* and is not a comprehensive mosquito species list for either country. *An.=Anopheles*, *Cx.=Culex*, *Lt.=Lutzia*, *Or.=Orthopodomia*, *Tr.=Tripteroides*; LaCasse and Yamaguti (1950) listed *An. k. koreicus* and *An. k. edwardsi* which were grouped under *An. koreicus* with *edwardsi* being a synonym of *An. koreicus* (WRBU 2001).

[2]*Lutzia vorax* reported as *Culex vorax* in LaCasse and Yamaguti (1950) but elevated to *Lutzia* by Tanaka (2003). Similarly, *Or. anopheloides* was reported as *Or. nipponica*, but *Or. nipponica* became a synonmy of *Or. anopheloides* after Knight and Mattingly (1950).

[3]This study represents the first reported larval associations with *An. l. japonicus* in Korea.

[4]See larval association list (Figure XXVIII) in LaCasse and Yamaguti (1950).

their dispersion to lower elevations during heavy rains and flooding.

At a site near Geumju Mountain, Gyeonggi Province, a series of small concrete dams had been built across an intermittent mountain stream. The upper side of these dams had become filled with gravel and dirt, but the lower sides had been washed out from the spillover during periods when the stream was flowing. These washed-out areas collected water and falling deciduous leaves during the non-flowing times when relatively large numbers of *An. l. japonicus* larvae were collected.

In another instance, *An. l. japonicus* larvae were collected from a red plastic container (KSK-31B(~1 liter), and presumably filled with rainwater) located along Highway 56 east of Sachang-ri, Hwacheon-gun, Gangwon Province, and about 75 m from a stream from which other culicids were collected from rock holes.

For sources that reported collection dates in Korea, *An. l. japonicus* were collected between May and October, with Whang (1962) and Hong and Ree (1968) reporting adult collections in May, and all other sources reporting adult and/or larval collections from July through October. These data suggest that peak *An. l. japonicus* populations may occur after the monsoon season, which normally occurs sometime during late June and early July as pools of water become more abundant.

Larvae developing on the edge of creeks and streams may potentially be washed downstream during the monsoon season, but after the rains stop, larval habitat would be plentiful allowing larvae to complete their development and for the succession of several broods. During intermittent moderate to heavy rains (typhoons, etc.) following the rainy monsoons, larvae from these stream margins and pools may be washed downstream. However, *An. l. japonicus* larvae have key structures, the modified setae 3-X, which allows them to stay anchored in currents, so the impact on larval and pupal development and dispersal caused by the monsoon rains and subsequent intermittent heavy rains are not understood. The modification is that the tips of the branches on seta 3-X are strong and hook-shaped, allowing the larvae to hang onto substrate within the current. These are located at the dorsal apex of segment X just beyond the saddle margin. The 3-X setae are paired and are the more apical of the two paired setae found there. The 3-X setae are just posterior to the 2-X setae which also have long branches. Usually the 3-X setae are very long and extend posteriorly, and are equal to or longer than the length of the anal papillae. This characteristic is shown in LaCasse and Yamaguti (1950, plate IV), Harrison and Scanlon (1975, Figures 36 and 48), and Harrison (1980, on all of the larvae in Myzomyia Series of subgenus *Cellia* of *Anopheles* figured in that book).

In another study, Kim et al. (2005) reported that they did not find *An. l. japonicus* on Jeju Island during their surveys in September-October 2003 and in June 2004 even though an attempt was made to collect them.

In Japan, LaCasse and Yamaguti (1950) regularly found *An. l. japonicus* from May through October in the

vicinity of Kyoto, Honshu. They also reported collecting large numbers of this species in mid-winter, even though they earlier indicated that the primary season appears to be May through October. They present a figure (Figure XXVII in their study) that shows that *An. l. japonicus* larvae were collected each month for an entire year.

Additionally, the U.S. Army 406th Medical Laboratory (1963) reported the collection of one overwintering female of this species in Tokyo in March 1963 during collection efforts from November 1962 to April 1963. Mogi (1996) also reported finding *An. l. japonicus* larvae of all instars to be present throughout the winter on Tanegashima and Yakushima Islands in Japan. These data indicate that at least in portions of Japan, *An. l. japonicus* larvae as well as adult females overwinter during the winter months.

To determine the overwintering or early development of *An. l. japonicus* in Korea, a larval survey was made at a previously collected site, Mt. Cheonggye, Gyeonggi Province and at Jeju Island. At Mt. Cheonggye (March 3, 2007), previously collected rock pools next to the flowing creek were sampled, and no larvae of any culicid species were collected. It was also noted that ice was still present in the shaded areas along the creek. On Jeju Island (April 3-10, 2007), six sites along creeks with rock holes and ranging from 30-1,000 m in elevation were sampled. Only 1st and 2nd instars of *Ochlerotatus togoi* Theobald larvae were found at three of these sites. While these two surveys do not rule out the possibility of *An. l. japonicus* overwintering as larvae in Korea, there have been no reported collections in Korea of overwintering larvae or adults outside of the May through October season reported earlier.

Tokyo is similar in latitude to Daegu in Korea, and Tanegashima and Yakushima Islands are approximately 3° latitude further south than Jeju Island, which represents the southernmost province in Korea. On latitude alone, the area from Daegu south through Jeju Island is bracketed by the overwintering findings for this species in Japan. This area in Korea has not been intensively studied for this species; therefore, further studies on this species are warranted and may provide insight into the overwintering status of *An. l. japonicus* in Korea.

The results of the molecular species identification for *Anopheles l. japonicus* were that the Universal forward primer, ITS2F (5'-TGTGAACTGCAGGACACATGAA-3'), binds to the same position on the ITS2 DNA for *An. lindesayi japonicus*, ITS2R (5'-ATGCTTAAATTTAGGGGGTAGTC-3'), producing a single amplicon (GenBank accession number, DQ398773).

Acknowledgments

The authors thank Jong Ho Shin, Soh Keum Lee, Kyle Clanton, Stephen Deutsch, and Walter Hampton for their assistance in the collection of the new specimens reported in this study. We also thank Ms. Suk Hee Yi for providing GIS support and mapping of collection sites. Funding for portions of this work was provided by the U.S. Department of Defense, Global Emerging Infections Surveillance and Response System, Silver Springs, MD, and the Armed Forces Medical Intelligence Center, Ft. Detrick, MD. The views expressed in this article are those of the authors and do not reflect the official policy or position of the Department of Army, the United States Air Force, the Department of Defense, or the U.S. Government.

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